

WHAT IS CLAIMED IS:

1. An isolated humanized antibody that immunoreacts with an epitope present on human TF and inhibits the binding of human coagulation factor VIIa to human TF.
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2. The isolated humanized antibody of claim 1, wherein the CDR amino acid sequences of the humanized antibody are derived from a parent monoclonal antibody.
3. The isolated humanized antibody of claim 2, wherein the parent monoclonal
10 antibody is a mouse monoclonal antibody.
4. The isolated humanized antibody of claim 1, wherein the humanized antibody is a Fab fragment.
- 15 5. The isolated humanized antibody of claim 1, wherein the humanized antibody is a F(ab)₂ fragment.
6. The isolated humanized antibody of claim 1, wherein the humanized antibody is a F(ab')₂ fragment.
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7. The isolated humanized antibody of claim 1, wherein the humanized antibody is a single chain Fv fragment.
8. The isolated humanized antibody of claim 1, wherein the humanized
25 antibody has a K_d for binding to human TF of from about 10⁻¹⁵ to about 10⁻⁸ M.
9. The isolated humanized antibody of claim 8, wherein the humanized antibody has a K_d for binding to human TF of from about 10⁻¹⁵ to about 10⁻¹⁰ M.
- 30 10. The isolated humanized antibody of claim 9, wherein the humanized antibody has a K_d for binding to human TF of from about 10⁻¹⁵ to about 10⁻¹² M.
11. The isolated humanized antibody of claim 1, wherein the human framework amino acid sequences of the humanized antibody are derived from a human antibody that
35 immunoreacts with a second epitope present on human TF.

12. The isolated humanized antibody of claim 11, wherein the second epitope comprises an amino acid residue at a particular position of human TF that also is comprised within the epitope.

5 13. The isolated humanized antibody of claim 12, wherein the amino acid residue at a particular position of human TF is selected from the group consisting of Trp45, Lys46, and Tyr94.

10 14. The isolated humanized antibody of claim 11, wherein the epitope or second epitope comprises an amino acid residue selected from the group consisting of Trp45, Lys46, and Tyr94.

15 15. A pharmaceutically acceptable composition comprising a therapeutically effective amount of the isolated humanized antibody of claim 1.

16. A pharmaceutically acceptable composition comprising a therapeutically effective amount of the isolated humanized antibody of claim 12.

20 17. A method for treating a FVIIa/TF related disorder in a human comprising administering a therapeutically effective amount of a humanized antibody that immunoreacts with an epitope on human TF and inhibits the binding of human coagulation factor VIIa to human TF.

25 18. A method for preparing a humanized antibody comprising preparing humanized antibodies against human TF and (i) testing the antibodies in a FVIIa/TF amidolytic assay and selecting a humanized antibody that inhibits TF-induced FVIIa amidolytic activity with an IC_{50} value that is less than the IC_{50} value of FFR-rFVIIa + about 100 nM (using 10 nM FVIIa in the assay), (ii) testing the antibodies in a FVIIa competition assay and selecting a humanized antibody that compete with FVIIa binding, or (iii) testing the
30 antibodies in a FVIIa signaling assay and selecting a humanized antibody that inhibits FVIIa-induced intracellular signaling.

35 19. The method of claim 21, wherein the method comprises testing antibodies in a FVIIa/TF amidolytic assay and selecting a humanized antibody that inhibits TF-induced FVIIa amidolytic activity with an IC_{50} value less than the IC_{50} value of FFR-rFVIIa + about 100 nM (using 10 nM FVIIa in the assay).

20. The method of claim 18, wherein the method comprises testing antibodies in a FVIIa competition assay and selecting a humanized antibody which competes with FVIIa binding.

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21. The method of claim 18, wherein the method comprises testing antibodies in a FVIIa signaling assay and selecting a humanized antibody that inhibits FVIIa-induced intracellular signaling.

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22. A method of preparing a humanized antibody comprising:

(a) preparing immortal cells that secrete humanized antibodies;

(b) isolating culture medium from the immortal cells comprising humanized antibodies,

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(c) testing the antibodies in (i) an indirect TF ELISA assay comprising TF in solution and selecting a humanized antibody therefrom that detectably immunoreacts with human TF in solution; (ii) a FVIIa competition assay and selecting a humanized antibody therefrom that detectably competes with FVIIa binding; (iii) a FVIIa/TF amidolytic assay and selecting a humanized antibody therefrom that inhibits TF-induced FVIIa amidolytic activity with an IC_{50} value that is less than the IC_{50} value of FFR-rFVIIa + about 100 nM (using 10 nM FVIIa in the assay); (iv) a FXa generation assay and selecting a humanized antibody therefrom that inhibits FXa generation with an IC_{50} value that is less than the IC_{50} value of FFR-rFVIIa + about 100 nM (using 0.1 nM FVIIa in the assay); (v) a TF-induced clot assay and selecting a humanized antibody therefrom that inhibits clot formation with an IC_{50} value that is less than the IC_{50} value of FFR-rFVIIa + about 1 nM; or (vi) any combination of (i)-(v) and selecting a humanized antibody that meets the criteria of the selected tests,

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(d) cultivating a cell that produces the humanized antibody, and

(e) isolating the humanized antibody.

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23. The method of claim 22, wherein the method comprises testing the antibodies in a direct TF ELISA assay comprising immobilized TF and selecting humanized antibodies that immunoreact with immobilized human TF.

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24. The method of claim 23, wherein the method further comprises testing antibodies in a FXa generation assay on a TF expressing cell and selecting a humanized antibody that inhibits FXa generation on TF expressing cell with an IC_{50} value less than the IC_{50} value of FFR-rFVIIa + about 500 nM (using 1 nM FVIIa in the assay).

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25. The method of claim 22, wherein the method comprises testing the antibodies in a whole cell TF binding assay and selecting a humanized antibody that competes with FVIIa binding to human TF expressed on the surface of the whole cells.

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26. The method of claim 22, wherein the method comprises testing the antibodies in a biosensor assay and selecting a humanized antibody with a K_d for binding to human TF of less than about 100 nM..

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27. The method of claim 22, wherein the method comprises testing the antibodies in a FVIIa signaling assay and selecting a humanized antibody that inhibits FVIIa-induced intracellular signaling.

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28. The method of claim 22, wherein the method comprises testing the antibodies in an epitope mapping assay and selecting a humanized antibody that immunoreacts with one or more selected epitopes on TF.

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29. The method of claim 28, wherein the preferred epitope comprises an amino acid residue selected from the group consisting of Trp45, Lys46, and Tyr94.

30. A cell that produces a humanized antibody that immunoreacts with an epitope on human TF and inhibits the binding of human coagulation factor VIIa to human TF.

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31. The cell of claim 30, wherein the cell is a mammalian cell.

32. The cell of claim 30, wherein the cell is a selected from the group consisting of CHO, BHK, HEK293, P3X63-Ag8, P3X63-AG8.653, PERC6, NS0, YB2/0, P3/NS1-Ag4-1 (NS-1), Sp2/0-Ag14 and S194/5.XXO.Bu.1.

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33. An isolated humanized antibody that immunoreacts with an epitope on a protein, wherein the human framework amino acid sequences of the humanized antibody are derived from a human antibody that immunoreacts with a second epitope on the protein and the second epitope comprises an amino acid residue at a particular position of the protein that also is comprised within the epitope.

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